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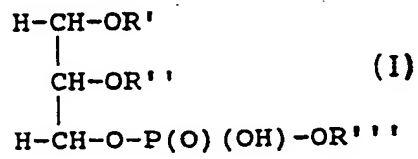

(I)

(57) Abstract

The invention relates to a powdery preparation for intranasal administration of a physiologically active agent containing: a) a lower alkyl ether of cellulose selected from the group consisting of methyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, and hydroxypropylmethyl cellulose, b) a cyclodextrin or a derivative thereof, and c) a phospholipid of general formula (I), wherein R' and R'' are each alkyl or alkylcarbonyl containing from 4 to 12 carbon atoms and R''' is 2-(trimethylammonio)ethyl, and optionally excipients such as a buffer or a binder. Such preparation shows an improved absorption.

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A COMPOSITION COMPRISING A PEPTIDE FOR NASAL ADMINISTRATION

The present invention relates to novel pharmaceutical preparations adapted for intranasal administration and to a process for preparing such preparations.

BACKGROUND OF THE INVENTION

While non-invasive medication, such as oral or rectal administration of a drug is undoubtedly most convenient to the patient, parenteral drug delivery is usualluy regarded as being the most effective. In particular, drugs which are inactivated in or poorly absorbed by the gastrointestinal tract and drugs which are subject to extensive first pass hepatic metabolism following oral administration are usually administered parenterally.

There are obvious inconveniences associated with parenteral drug administration, such as the need for sterile delivery devices, pain and irritation caused by reiterated injections and the potential risk of infection. Therefore, alternative means of drug delivery, equalling parenteral administration in the sense that first pass metabolism is circumvented, have been sought. One such potentically promising alternative is drug administration via the nasal route. However, just as is the case with other methods for non-invasive medication, the bioavailability of a drug after intranasal administration is largely unpredictable, depending inter alia on the chemical nature of the drug.

Thus it is known that progesterone and propranolol are absorbed from the nasal cavity in a manner providing blood levels almost equal to interveneous administration.

Other examples of intranasal formulations of pharmaceutically active agents with molecular weights up to about 1 kD are known, for example compositions containing ergopeptide alkaloids dissolved in aqueous ethanol administered as aerosols

(Swiss Patent No. 636,011), salts of pharmaceutically active amines with fatty acids (Canadian Patent No. 988,852) and catecholamine suspended in a fatty acid (or ester) emulsified with polyoxyethylene (European Patent Publication No. 0 160 501 A).

Over the last decades a variety of (mainly synthetic) polypeptide drugs have been developed. In general, polypeptides have been administered parenterally due to incomplete absorption from a digestive instability in the alimentary canal. This is probably the reason why in particular studies of the nasal delivery of polypeptides have been intensified during recent years. It has been found that while some smaller polypeptides (op to about 10 amino acids residues) may be reasonably well absorbed intranassally from simple aqueous formulations, generally the nasal bioavailability of larger polypeptides becomes both incomplete and variable, and increasingly so with increasing molecular weight (for review, see L. Illum: Archiv for Pharmaci og Chemi 94 (1987), 127-135.

With a view to overcoming the disadvantages encountered particularly with nasal delivery compositions containing larger polypeptides, the additional incorporation of a variety of biocompatible absorption promoting agents of so-called enhancers has been devised.

In the respect reference is made to European Patent Publication No. 0 111 841 A, disclosing the absorption enhancing effect of a bile acid and to U.S. Patent No. 4,476,116, using chelating agents such as EDTA.

Nasal formulations adapted to growth hormone delivery would naturally be highly preferred by the patient who has to be given growth hormone by many administrations to the presently available preparations for parenteral administration, provided that the growth hormone is absorbed to a reasonably effective and constant extent from the nasal cavity. A variety of absorp-

tion enhancing agents, mainly surfactants, have been devised for nasal formulations.

Ionic as well as non-ionic surfactant enhancers, such as bile acid salts and polyoxyethylene higher alcohol ethers are disclosed in British Patent No. 1,527,605 while the use of a specific polyoxyethylene higher alcohol ether, namely polyoxyethylene-9 lauryl ether is described in: R. Salzman et al., New England J. of Med. 312 (1985), 1078-1084. Other enhancers, for example salts of taurodihydrofusidic acid, are disclosed in U.S. Patent No. 4,548,922.

The chemical structure of enhancers known heretofore deviate considerably from those of known constituents of cellular membranes, including those of the nasal cavity. This feature could possibly explain their general proneness to cause nasal irritation or even permanent damage to the nasal membrane, particularly during chronic administration.

The use of phospholipids such as phosphatidylcholines (lecitins) as an enhancer for nasal administration of in particular insulin is disclosed in International Patent Publication No. WO88/04556.

Furthermore, it has been proposed to utilize α-cyclodextrin to increase the absorbability of a hydrophilic drug, vide European Patent Publication No. 94 157A.

Still further is it disclosed in European Patent Publication No. 23 359 to treat the mucosa of the nasal cavity using a powdery pharmaceutical preparation comprising a lower alkyl ether of cellulose having a specified viscosity.

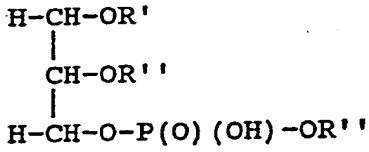
In spite of all these attempts to find suitable pharmaceutical preparations for mucosal or nasal administration there is still a need for a powdery nasal pharmaceutical preparation which is suitable for systemic treatment using larger polypeptide pharmaceuticals such as insulin and insulin derivatives,

proinsulin, glucagon, parathyroid hormone, parathyroid hormone antagonist, calcitonin, vasopressin, renin, prolactin, growth hormone, thyroid stimulating hormone, corticotropin, corticotropin-releasing factor, follicle stimulating hormone, luteinizing hormone, chorionic gonadotropin, atrial peptides, interferon, tissue plasminogen activator, gammaglobulins, Factor VII, Factor VIII, growth hormone releasing hormone, luteinizing hormone releasing hormone, somatostatin and cholecystokinins.

It has now surprisingly been found that the absorption from powdery preparations is increased when using the preparations of the present invention meeting one of the critical requirements for nasal administration of essential polypeptide pharmaceuticals, i.e. that a sufficient amount of the preparation is reproducibly absorbed enabling a reliable administration of a given dosis of the preparation.

The present invention relates to a powdery preparation for intranasal administration of a physiologically active agent, said preparation being characterized by containing

a) a lower alkyl ether of cellulose selected from the group consisting of methyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, and hydroxypropylmethyl cellulose,
b) a cyclodextrin or a derivative thereof, and
c) a phospholipid of the general formula



wherein R' and R'' are each alkyl or alkylcarbonyl containing from 4 to 12 carbon atoms and R''' is 2-(trimethylammonio)ethyl, and optionally excipients such as a buffer and/or a binder.

The preparation of the invention preferably contains, as the lower alkyl ether of cellulose, hydroxymethylpropyl cellulose (HPMC) or methyl cellulose (MC).

The contents of lower alkyl ether of cellulose is normally in the range from 25% w/w to 80% w/w, e.g. 30-80% w/w, especially 30-75% w/w of the preparation.

A cyclodextrin or a derivative thereof may e.g. be α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, hydroxypropylated, hydroxyethylated, ethylated or methylated derivatives thereof, branched cyclodextrins or cyclodextrin polymers.

As the cyclodextrin the preparation of the invention preferably contains α-cyclodextrin.

The contents of a cyclodextrin is normally in the range from 2% w/w to 60% w/w, preferably in the range 5-45% w/w of the preparation.

In the general formula for the phospholipid "alkyl" or "alkylcarbonyl" containing from 4 to 12 carbon atoms are considered to comprise liniear and branched alkyl and alkylcarbonyl such as n-butyl, sec.butyl, isobutyl, tert.butyl, n-pentyl, isopentyl, neopentyl, n-hexyl, isohexyl, 1-, 2-, or 3-ethyl-butyl, 1- or 2-propyl-propyl- and 1-butyl-ethyl, n-heptyl, isoheptyl, n-octyl, isooctyl, n-nonyl, isononyl, n-decyl, n-undecyl or n-dodecyl, or the corresponding n-propylcarbonyl, isopropylcarbonyl, sec.propylcarbonyl, n-butylcarbnyl, sec.butylcarbonyl, isobutylcarbonyl, tert.butylcarbonyl, n-pentylcarbonyl, isopentylcarbnonyl, neopentylcarbonyl, n-hexylcarbonyl, isohexylcarbonyl, 1-, 2-, or 3-ethylbutylcarbonyl, 1- or 2-propylpropylcarbonyl, 1-butylethylcarbonyl, n-heptylcarbonyl, isoheptylcarbonyl, n-octylcarbonyl, isooctylcarbonyl, n-nonylcarbonyl, isononylcarbonyl, n-decylcarbonyl or n-undecylcarbonyl.

The phospholipid contained in the preparation of the invention is preferably a lecitin, more preferred didecanoyl L-α-phosphatidylcholine.

The contents of phospholipid is normally in the range from 2% w/w to 20% w/w, e.g. in the range 4-20% w/w, preferably in the range 6-18% w/w of the preparation.

The physiologically active agent to be administered in the preparation of the invention is preferably a polypeptide.

The preferred polypeptides to be incorporated in the preparations of the invention are growth hormone, preferably human growth hormone, or a derivative or an analogue thereof, insulin or a derivative or an analogue thereof, calcitonin or glucagon or a derivative or an analogue thereof. These polypeptides may be derived from a natural source, e.g. by extraction from pancreas or pituitary glands, or be prepared by chemical synthesis or by recombinant techniques.

More preferred preparations of the invention comprise human growth hormone, insulin, calcitonin or glucagon, or a derivative or an analogue thereof such as methionyl growth hormone.

Especially preferred is a preparation of the invention comprising human growth hormone as is enables a reliable nasal administration in a preparation in which the human growth hormone is stable.

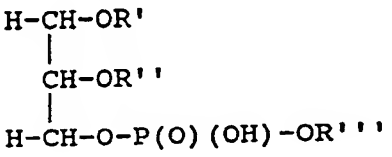
A buffer optionally being present buffers the pH to a value in the physiological range from 4.0 to 9.0, more preferred in the range of 6.5-7.5, most preferred about 7.2. The buffer may for example be an amino acid such as glycine or glycylglycine, phosphate buffer, citrate buffer, or acetate buffer. Preferred buffers are glycine and citrate buffer. The most preferred buffer is a combination of glycine and citric acid.

A further preferred aspect of the invention is a preparation comprising human growth hormone,

a) a lower alkyl ether of cellulose selected from the group consisting of methyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, and hydroxypropylmethyl cellulose,

b) a cyclodextrin or a derivative thereof, and

c) a phospholipid of the general formula



wherein R' and R'' are each alkyl or alkylcarbonyl containing from 4 to 12 carbon atoms and R''' is 2-(trimethylammonio)ethyl, and wherein the cyclodextrin is α-cyclodextrin, and further comprising glycine and citric acid. The use of glycine and citrat together with α-CD allows for the omission of mannitol normally necessary for lyophilization but giving rise to significantly lower nasal absorption. Furthermore, α-CD and glycine and citrate give a good stability. Still further, this formulation is fully acceptable for the patients having no adverse effects from nasal administration thereof.

The contents of buffer is normally in the range of from 0.1 to 5% w/w of the preparation.

The preparation of the invention may optionally comprise a separate bulking agent for the lyophilization. Such bulking agent may be a water soluble macromolecular substance such as hydrolysed gelatine or dextran.

A binder optionally being present in the preparation may for example be ethyl cellulose or polyvinylpyrrolidone.

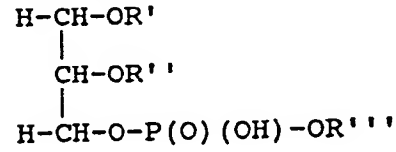
The present invention also relates to a method for preparing a powdery preparation for intranasal administraion of a physiologically active agent containing

a) a lower alkyl ether of cellulose selected from the group consisting of methyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, and hydroxypropylmethyl cellulose,

b) a cyclodextrin or a derivative thereof, and

b) a cyclodextrin or a derivative thereof, and

c) a phospholipid of the general formula



wherein R' and R'' are each alkyl or alkylcarbonyl containing from 4 to 12 carbon atoms and R''' is 2-(trimethylammonio)ethyl for the preparation of a pharmaceutical preparation for intranasal administration of a physiologically active agent.

Such use is of particular interest for the the preparation of nasal preparations comprising a peptide as stated above, preferably for the preparation of powdery nasal preparations comprising human growth hormone or a derivative thereof.

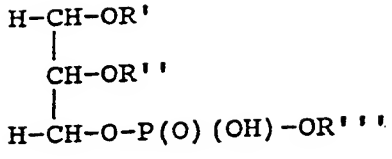
The preparations of the invention may be used to treat all conditions for which human growth hormone is indicated, e.g. dwarfism, short stature, Turner's syndrome, intoxicated individuals, individuals suffering from subnormal or absent fertility, or substitution therapy for adults, e.g. adult dwarfs, or individuals having had hypophysectomy or suffering from chronic renal illness or failure.

The Invention also relates to a process for treating growth hormone deficiency in higher mammals comprising administering to the individual, via the nasal route, a sufficient amount of growth hormone in the form of a powdery preparation for intranasal administration of a physiologically active agent containing

a) a lower alkyl ether of cellulose selected from the group consisting of methyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, and hydroxypropylmethyl cellulose,

b) a cyclodextrin or a derivative thereof, and

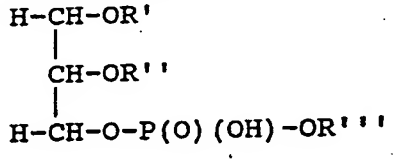
c) a phospholipid of the general formula



wherein R' and R'' are each alkyl or alkylcarbonyl containing from 4 to 12 carbon atoms and R''' is 2-(trimethylammonio)ethyl, and optionally excipients such as a buffer or a binder.

In a further aspect, the invention relates to a process for normalizing the growth hormone blood levels in a higher mammal suffering from growth hormone deficiency comprising administering to the mammal via the nasal route a sufficient amount of growth hormone in the form of a powdery preparation for intranasal administration of a physiologically active agent containing

a) a lower alkyl ether of cellulose selected from the group consisting of methyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, and hydroxypropylmethyl cellulose,
b) a cyclodextrin or a derivative thereof, and
c) a phospholipid of the general formula

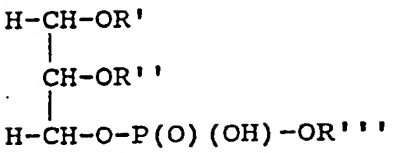


wherein R' and R'' are each alkyl or alkylcarbonyl containing from 4 to 12 carbon atoms and R''' is 2-(trimethylammonio)ethyl, and optionally excipients such as a buffer or a binder.

In a still further aspect, the invention related to a method of administering human growth hormone comprising the steps of: administering a formulation with an aerosol device wherein the formulation comprises

a) human growth hormon
b) a lower alkyl ether of cellulose selected from the group consisting of methyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, and hydroxypropylmethyl cellulose,
c) a cyclodextrin or a derivative thereof, and

d) a phospholipid of the general formula



wherein R' and R'' are each alkyl or alkylcarbonyl containing from 4 to 12 carbon atoms and R''' is 2-(trimethylammonio)ethyl, and optionally excipients such as a buffer or a binder.

The invention is described more in detail with reference to the drawings in which

Fig. 1 shows the improved plasma levels of hGH in rabbits after administration of a preparation of the invention,

Fig. 2 shows the serum profiles of the same preparations as in Fig. 1, administered to human beings,

Fig. 3 shows the improved serum profiles of another product of the invention in human beings,

Fig. 4 shows the serum profiles of hGH after administration of various preparations of the invention to human beings, and

Fig. 5 shows the serum profiles of hGH after administration of a preparation of the invention to growth hormone deficient human beings.

The inventions is further explained below with reference to the Examples describing experiments carried out to support the invention. The examples are to be considered as explaining the invention and not as limiting the invention, the scope of which is set forth in the appended claims.

EXAMPLES

Example 1

Nasal powder A

Composition per 20 mg:

hGH	2.05 mg ~ 6 IU
Didecanoylphosphatidylcholine (DDPC)	1.5 mg
α-cyclodextrin (α-CD)	16.0 mg
Glycine	0.1 mg
Citric acid	0.2 mg

a) 126 mg DDPC was dispersed in 3.0 ml sterile water in a ultrasound batch. (b) 981 mg α-CD was dissolved in 8.1 mg sterile water. (c) 6.15 g of a solution containing 22.80 mg hGH/ml in glycine/citrate buffer pH 7.2 were mixed with 2.40 g a) and solution b). The mixture was lyophilized, blended and sieved through a 0.3 mm sieve. 20 mg powder was weighed into Finn-Tips no. 60 and closed with Parafilm® (American Can Company) and stored at 4°C in a desiccator until use.

Nasal powder B

Composition per 20 mg:

hGH	2.05 mg ~ 6 IU
Didecanoylphosphatidylcyholine (DDPC)	3.2 mg
α-cyclodextrin (α-CD)	14.7 mg

a) 885 mg α-CD was dissolved in 7.3 sterile water and mixed with 6.5 g of a solution containing 19.31 mg hGH/ml. (b) 185 mg DDPC was dissolved in 275 µl ethanol 96%. (c) 924 mg lyophilized powder was mixed in a mortar and granulated with solution b). After sieving through 0.7 mm and 0.3 mm sieves, the powder was vacuum dried in a desiccator for 48 hours. 20 mg powder was weighed into Finn-Tips no. 60 and closed with Parafilm® and stored at 4°C in a desiccator until use.

Nasal powder C

Composition per 20 mg

B-hGH	2.05 mg ~ 6 IU
Didecanoylphosphatidylcholine (DDPC)	2.0 mg
α-cyclodextrin (α-CD)	7.9 mg
Methocel® E4M (hydroxypropylmethylcellulose)	7.9 mg
Glycine	0.1 mg
Citric acid	0.2 mg

a) 479 mg α-CD was dissolved in 4.0 mg sterile water and mixed with 12.95 g of a solution containing 10.9 mg hGH/ml in glycine/citrate buffer pH 7.2. The solution was lyophilized. (b) 115 mg DDPC was dissolved in 275 µl ethanol 96%. (c) In a mortar 551 mg lyophilized powder was mixed with 439 mg Methocel® E4M and granulated with b). The further procedure was analogous to that of preparation B.

Example 2

6 IU hGH in nasal powder A, B and C were dosed in New Zealand male rabbits. Each nasal powder was given to 6 animals, and one group af 3 animals was given 0.075 IU/kg b.wt intravenously. The rabbits were fixed in holders, and catheters were inserted in the ear artery for blood sampling. A special syringe with well defined air pressure was used to blow the powder into the nostrils.

From animals receiving nasal administration 0.5 ml blood was sampled into ice cold heparinized tubes (250 IU/ml) at ÷5, 10, 20, 30, 40, 50, 60, 75, 90, 120, 180, and 240 minutes, and from animals receiving i.v. administration at ÷5, 1, 6, 10, 20, 30, 40, 50, 60, 75, 90, and 120 minutes.

Plasma was analyzed with respect to immunoreactive hGH using a hGH-ELISA method.

The results are shown in Figure 1 and the below Table I.

Table I

Nasal powder	AUC (ng/ml x min.)	C_{max} (ng/ml)	% bioavailab.
A	25748	276	17.4
B	39117	453	26.0
C	20223	158	12.8

Example 3

12 IU hGH in the powdery compositions A, B, and C, respectively from example 1 were administered intranasally to 8 healthy volunteers (age 20-30 years). Each subject was given 6 IU in each nostril. The pipette point was mounted on a plastic syringe and the powder were blown into the nostril by a specific quantity of precompressed air released from the syringe.

Blood samples for determination of hGH were taken at ÷5, 0, 5, 10, 15, 20, 25, 30, 45, 75, 90, 105, 120, 150, and 180 min. The blood samples were assayed for GH using a RIA method. The preparations were compared with respect to C_{max}, and AUC of the plasma profiles by means of analysis of variance.
The results are shown in Table II and Figure 2.

Table II

Mean values (n=8)

Nasal powder	AUC (ng/ml x min)	C_{max}(ng/ml)
A	537	6.7
B	691	7.7
C	1522	14.1

Powder C gave significantly larger AUC and C_{max} than powder A and B not containing HPMC. The highest absorption was found for the composition (nasal powder C) containing both α-CD and HPMC.

Example 4

Nasal powder D

Composition per 20 mg:

B-hGH	2.05 mg ~ 6 IU
Didecanoylphosphatidylcholine (DDPC)	1.6 mg
α-cyclodextrin (α-CD)	4.0 mg
Methocel® E4M (hydroxypropylmethylcellulose)	12.4 mg
Glycine	0.1 mg
Citric acid	0.2 mg

(a) 240 mg α-CD was dissolved in 2.0 g sterile water and mixed with 8.2 g of a solution containing 16.27 mg hGH/ml in glycine-/citrate buffer pH 7.2. The solution was lyophilized. (b) 93 mg DDPC was dissolved in 275 µl ethanol 96%. (c) In a mortar 333 mg lyophilized powder was mixed with 683 mg Methocel® E4M. The procedure was similar to that of preparing nasal powder B.

Nasal powder E

Composition per 20 mg:

B-hGH	2.05 mg ~ 6 IU
Didecanoylphosphatidylcholine (DDPC)	1.6 mg
Avicel® PH 101	4.0 mg
Methocel® E4M	12.4 mg
Glycine	0.1 mg
Citric acid	0.2 mg

(a) 240 mg Avicel® PH 101 was suspended for 2 hours in 2.5 mg sterile water and mixed with 8.2 g of a solution containing 16.27 mg hGH/ml in glycine/citrate buffer pH 7.2. The mixture was lyophilized. The subsequent procedure was similar to the preparation of nasal powder D.

Example 5

12 IU hGH in the two nasal powders D and E from example 3 were administered intranasally to 8 healthy volunteers. The experiment was carried out in the same manner as described in example 3. The results are shown in Figure 3 and Table III.

Table III

Mean values (n=8)

Nasal powder	AUC (ng/ml x min.)	C_{max} (ng/ml)
D	1075	11.3
E	639	6.3

Powder D gave larger AUC and C_{max} than powder E, which contained no α-CD. Again the highest absorption was found from the powder containing both α-CD and HPMC.

Example 6

Nasal powder F

Composition per 20 mg

B-hGH	2.05 mg ~ 6 IU
Didecanoylphosphatidylcholine (DDPC)	1.6 mg
α-cyclodextrin (α-CD)	8.2 mg
Natrosol® Hx Pharm 250 (hydroxyethylcellulose)	8.2 mg
Glycine	0.1 mg
Citric acid	0.2 mg

(a) 492 mg α-CD was dissolved in 4.2 g sterile water and mixed with 7.3 g of a solution containing 16.92 mg hGH/ml in glycine-/citrate buffer pH 7.2. The solution was lyophilized. (b) 84 mg DDPC was dissolved in 275 µl ethanol 96%. (c) In a mortar 513 mg lyophilized powder was mixed with 410 mg Natrosol® Hx Pharm 250. The subsequent procedure was similar to the preparation of nasal powder B in Example 1.

Nasal powder G

Composition per 20 mg

B-hGH	2.05 mg ~ 6 IU
Didecanoylphosphatidylcholine (DDPC)	1.6 mg
α-cyclodextrin (α-CD)	8.2 mg
Methocel® A4M (methylcellulose)	8.2 mg
Glycine	0.1 mg
Citric acid	0.2 mg

(a) The lyophilized powder was prepared in the same manner as described for nasal powder F. (b) 93 mg DDPC was dissolved in 275 µl ethanol 96%. (c) In a mortar 564 mg lyophilized powder was mixed with 451 mg Methocel® A4M. The subsequent procedure was similar to the preparation of nasal powder B in Example 1.

Nasal powder H

Composition per 13 mg:

B-hGH	2.05 mg ~ 6 IU
Didecanoylphosphatidylcholine (DDPC)	2.2 mg
α-cyclodextrin (α-CD)	4.2 mg
Methocel® E4M (hydroxypropylmethylcellulose)	4.2 mg
Glycine	0.1 mg
Citric acid	0.2 mg

(a) 384 mg α-CD was dissolved in 3.2 mg sterile water and mixed with 14.6 g of a solution containing 16.92 mg hGH/ml in glycine/citrate buffer pH 7.2.

The solution was lyophilized. (b) 185 mg DDPC was dissiolved in 275 µl ethanol 96%. (c) 578 mg lyophilized powder was mixed with 352 mg Methocel® E4M, and the subsequent procedure was similar to the preparation of nasal powder B except that 13 mg powder was weighed into Finn-Tips.

Example 7

The three nasal powders F, G, and H from Example 6 were tested in an amount corresponding to 12 IU in 8 healthy volunteers in the same manner as described in Example 3. The results are shown in Figure 4 and in Table IV (n=8).

Table IV

Mean values (n=8)

Nasal powder	AUC (ng/ml x min.)	C_{max} (ng/ml)
F	1115	10.44
G	1476	16.43
H	1694	16.93

Example 8

Nasal powder I

Composition per 20 mg:

hGH	1.023 mg - 3 IU
DDPC	1.6 mg
α-CD	17.1 mg
glycine	0.1 mg
citric acid	0.2 mg

The method of preparation was analogous to the preparation Nasal powder A. hGH was freeze dried in a solution containing α-CD, citrate and glycine. The derived powder was granulated with an ethanol solution of DDPC.

Nasal powder J

Composition per 20 mg

hGH	1.023 mg - 3 IU
DDPC	1.6 mg
mannitol	5.0 mg
α-CD	12.1 mg
glycine	0.1 mg
citric acid	0.2 mg

hGH was freeze dried in a solution of mannitol, glycine and citrate. The freeze dried powder were mixed with α-CD and granulated with an ethanol solution of DDPC.

Example 9

3 IU hGH in nasal powder I and J were dosed in male rabbits. The absorption of hGH was measured by the same procedure as described in example 2.

The results are shown in Table V

Table V

Nasal powder	AUC ng/ml x min.	C_{max} ng/ml	bioavailability %
I	23772	261	31.0
J	8604	62	8.5

Powder I comprising α-CD as bulking agent for lyophilization gave significantly larger AUC and C_{max} than powder J comprising mannitol as bulking agent for the lyophilization.

Example 10

Nasal powder K

Composition per 12 mg

hGH	2.05 mg ~ 6 IU
DDPC	1.92 mg
Methocel® E4M	3.69 mg
α-CD	3.69 mg
Ethocel	0.30 mg
glycine	0.15 mg
citric acid	0.22 mg

The preparation is made in an analogous manner as nasal powder H. The Ethocel was added to the ethanol containing DDPC.

12 mg powder was weighed into plastic capsules which were sealed with alu-foil in both ends.

The capsules were stored at 4°C and at 30°C. The chemical stability of hGH in the preparation was studied by HPLC-methods. The results appears from table VI showing that the preparation according to the invention may be considered as stable.

Table VI

Stability data on nasal powder K

	hGH mg/12 mg	hGH desamido forms %	hGH Polymer %	hGH Dimer %	hGH Oxidized forms %	Water %
Time = 0	1.83 1.82	2.1 2.4	<0.2 <0.2	0.8 1.1	1.7 1.9	
4 weeks, 30°C	1.94 1.90	3.4 3.5	<0.2 <0.2	1.0 0.9	1.3 1.8	
8 weeks, 30°C	1.87 1.85	3.0 3.3	<0.2 <0.2	1.3 1.5	1.9 2.0	
12 weeks, 30°C	2.02 1.89	3.9 3.9	<0.2 <0.2	1.1 1.5	2.4 2.4	3.6
12 weeks, 4°C	1.85 1.82	2.7 2.7	<0.2 <0.2	0.9 1.1	1.3 1.3	3.2
6 months, 4°C	1.83 1.90	2.6 2.6	<0.5 <0.5	0.3 0.2	1.2 1.4	3.2 3.0

Example 11

Six nasal powders having various contents of citrate were prepared by the procedure described for nasal powder H. The composition of the powders are shown in Table VII.

Table VII

Composition mg/20 mg	1	2	3	4	5
B-hGH	1.0	1.0	1.0	1.0	1.0
DDPC	1.6	1.6	1.6	1.6	1.6
Citrate	-	0.5	0.5	0.5	1.0
α-CD	8.7	8.45	8.45	8.45	8.2
Methocel E4M	8.7	8.45	8.45	8.45	8.2
Ethanol	99.9%	99.9%	99.9%	99.9%	99.9%

The contents of hGH dimer forms was determined by GPC before and after the granulation process. The results are shown in Table VIII.

Table VIII

Composition	Before gran.	After gran.	
	Dimer %	Dimer %	ΔDimer %
Bulk	1.0	-	-
1	2.4	8.4	+6.0
2	1.6	1.5	-0.1
3	1.6	3.4	+1.8
4	1.6	2.2	+0.6
5	1.1	3.1	+2.0

The results show, that citrate stabilises hGH during the processing.

Example 12

12 IU hGH in the powdery composition K from Example 10 were administered nasally to 7 growth hormone deficient adult patients. The experiment was carried out in the same mannar as described in example 3.

The results are shown in figur 5 and in the Table VIII.

Table VIII

Mean values (n=6)

Nasal powder	AUC (189 min) ng/ml x min	C_{max} (ng/ml)
K	1977	20.1

Powder K according to the invention gave an AUC and C_{max} comparable to that obtained in Example 3. The experiments demonstrates that the preparation of the invention is absorbed equally well in hGH deficient human beings.

Example 13

Nasal powder L

Composition per 12 mg:

hGH	2.05 mg ~ 6 IU
DDPC	0.96 mg
Methocel® E4M	4.65 mg
α-CD	3.69 mg
Ethocel	0.30 mg
glycine	0.15 mg
citric acid	0.22 mg

Nasal powder M

Composition per 12 mg:

hGH	2.05 mg ~ 6 IU
Methocel® E4M	5.61 mg
α-CD	3.69 mg
Ethocel	0.30 mg
glycine	0.15 mg
citric acid	0.22 mg

The preparations were made in analogous manner as Nasal Powder H.

The two nasal powders L and M were tested in an amount corresponding to 12 IU in 16 healthy volunteers in the same manner as described in Example 3.

The results are shown in Table IX.

Table IX

Mean value (n=16)

Nasal Powder	AUC (ng/ml x min)	C_{max} (ng/ml)
L	2769 (± 2075)	28.1 (±20.9)
M	1167 (± 683)	10.8 (± 6.5)

Powder L comprising DDPC gave a clearly higher AUC and C_{max} than powder M where DDPC was omitted.

Example 14

Nasal Powder N

Composition per 12 mg:

hGH	1.023 mg ~ 3 IU
DDPC	1.92 mg
Ethocel	0.30 mg
glycine	0.154 mg
citrate	0.215 mg
Methocel® E4M	4.71 mg
gelatine, hydr.	1.84 mg
α-CD	1.84 mg

Nasal Powder O

composition per 12 mg:

hGH	1.023 mg ~ 3 IU
DDPC	1.92 mg
Ethocel	0.30 mg
glycine	0.154 mg
citrate	0.215 mg
Methocel® E4M	5.79 mg
gelatine, hydr.	2.0 mg
α-CD	0.60 mg

A solution of pH=7.0 containing hGH, α-CD, citrate, glycine and hydrolysed gelatine was lyophilized. The powder was sieved and mixed with Methocel®. The powder mixture was wet massed with a solution of DDPC and Ethocel in ethanol. After sieving and drying the powder showed good free flowing properties giving an easy powder filling.

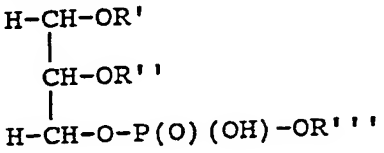
CLAIMS

1. A powdery preparation for intranasal administration of a physiologically active agent containing

a) a lower alkyl ether of cellulose selected from the group consisting of methyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, and hydroxypropylmethyl cellulose,

b) a cyclodextrin or a derivative thereof, and

c) a phospholipid of the general formula



wherein R' and R'' are each alkyl or alkylcarbonyl containing from 4 to 12 carbon atoms and R''' is 2-(trimethylammonio)ethyl, and optionally excipients such as a buffer or a binder.

2. A preparation as claimed in claim 1 wherein the lower alkyl ether of cellulose is hydroxymethylpropyl cellulose (HPMC) or methyl cellulose (MC), the cyclodextrin is α-cyclodextrin, and the phospholipid is a lecitin.

3. A preparation as claimed in claim 2 wherein the lecitin is didecanoyl L-α-phosphatidylcholine.

4. A preparation as claimed in claim 2 or 3 wherein the contents of lower alkyl ether of cellulose is 30-80% w/w, the contents of cyclodextrin is 5-45% w/w, and the contents of phospholipid is in the range of 2-20% w/w.

5. A preparation as claimed in any of claims 1-4 wherein the physiologically active agent is a polypeptide such as human growth hormone, insulin, calcitonin, and glucagon.

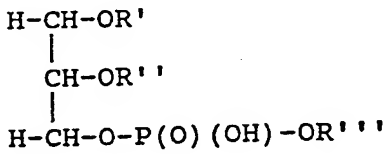
6. A preparation as claimed in claim 5 wherein the polypeptide is human growth hormone.

7. A method for preparing a powdery preparation for intranasal administraion of a physiologically active agent containing

a) a lower alkyl ether of cellulose selected from the group consisting of methyl cellulose, and hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose,

b) a cyclodextrin or a derivative thereof, and

c) a phospholipid of the general formula



wherein R' and R'' are each alkyl or alkylcarbonyl containing from 4 to 12 carbon atoms and R''' is 2-(trimethylammonio)ethyl, and optionally excipients such as a buffer or a binder comprising:

a) lyophilization of the physiological active agent and the cyclodextrin in a pH-buffered solution,

b) admixing the lower alkyl ether of cellulose to the lyophilized powder,

c) wet-massing the powder mixture with an ethanol solution of the phospholipid and a binder, and

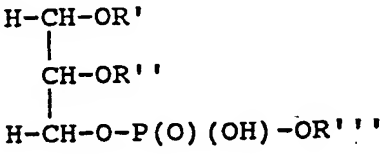
d) sieving and drying the mixture to generate the powder in granular form.

8. Use of a combination of

a) a lower alkyl ether of cellulose selected from the group consisting of methyl cellulose, and hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose,

b) a cyclodextrin or a derivative thereof, and

c) a phospholipid of the general formula



wherein R' and R'' are each alkyl or alkylcarbonyl containing from 4 to 12 carbon atoms and R''' is 2-(trimethylammonio)ethyl

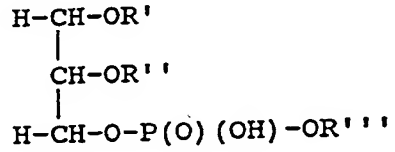
for the preparation of a pharmaceutical preparation for intranasal administration of a physiologically active agent.

9. A process for treating growth hormone deficiency in higher mammals comprising administering to the individual, via the nasal route, a sufficient amount of growth hormone in the form of a powdery preparation for intranasal administration of a physiologically active agent containing

a) a lower alkyl ether of cellulose selected from the group consisting of methyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, and hydroxypropylmethyl cellulose,

b) a cyclodextrin or a derivative thereof, and

c) a phospholipid of the general formula



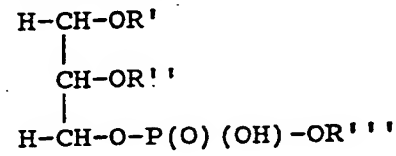
wherein R' and R'' are each alkyl or alkylcarbonyl containing from 4 to 12 carbon atoms and R''' is 2-(trimethylammonio)ethyl, and optionally excipients such as a buffer or a binder.

10. A process for normalizing the growth hormone blood levels in a higher mammal suffering from growth hormone deficiency comprising administering to the mammal via the nasal route a sufficient amount of growth hormone in the form of a powdery preparation for intranasal administration of a physiologically active agent containing

a) a lower alkyl ether of cellulose selected from the group consisting of methyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, and hydroxypropylmethyl cellulose,

b) a cyclodextrin or a derivative thereof, and

c) a phospholipid of the general formula



wherein R' and R'' are each alkyl or alkylcarbonyl containing from 4 to 12 carbon atoms and R''' is 2-(trimethylammonio)ethyl, and optionally excipients such as a buffer or a binder.

11. A method of administering human growth hormone comprising the steps of:

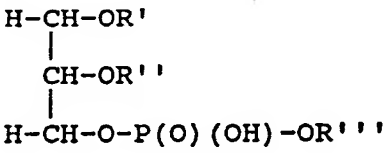
administering a formulation with an aerosol device wherein the formulation comprises

a) human growth hormon

b) a lower alkyl ether of cellulose selected from the group consisting of methyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, and hydroxypropylmethyl cellulose,

c) a cyclodextrin or a derivative thereof, and

d) a phospholipid of the general formula



wherein R' and R'' are each alkyl or alkylcarbonyl containing from 4 to 12 carbon atoms and R''' is 2-(trimethylammonio)ethyl, and optionally excipients such as a buffer or a binder.

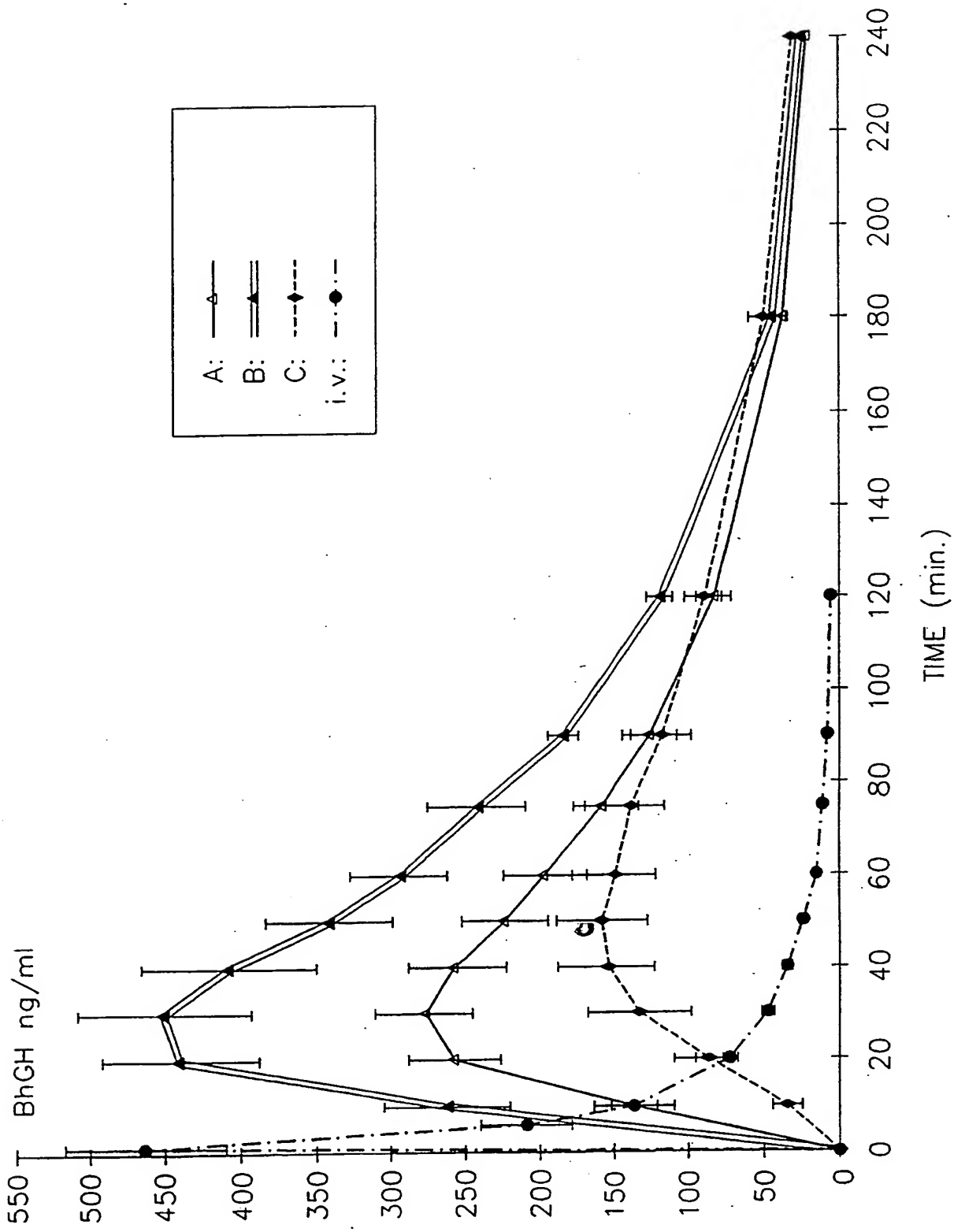

A:
B:
C:
i.v.:
BhGH ng/ml
550
500
450
400
350
300
250
200
150
100
50
0
0
20
40
60
80
100
120
140
160
180
200
220
240
TIME (min.)

Fig. 1

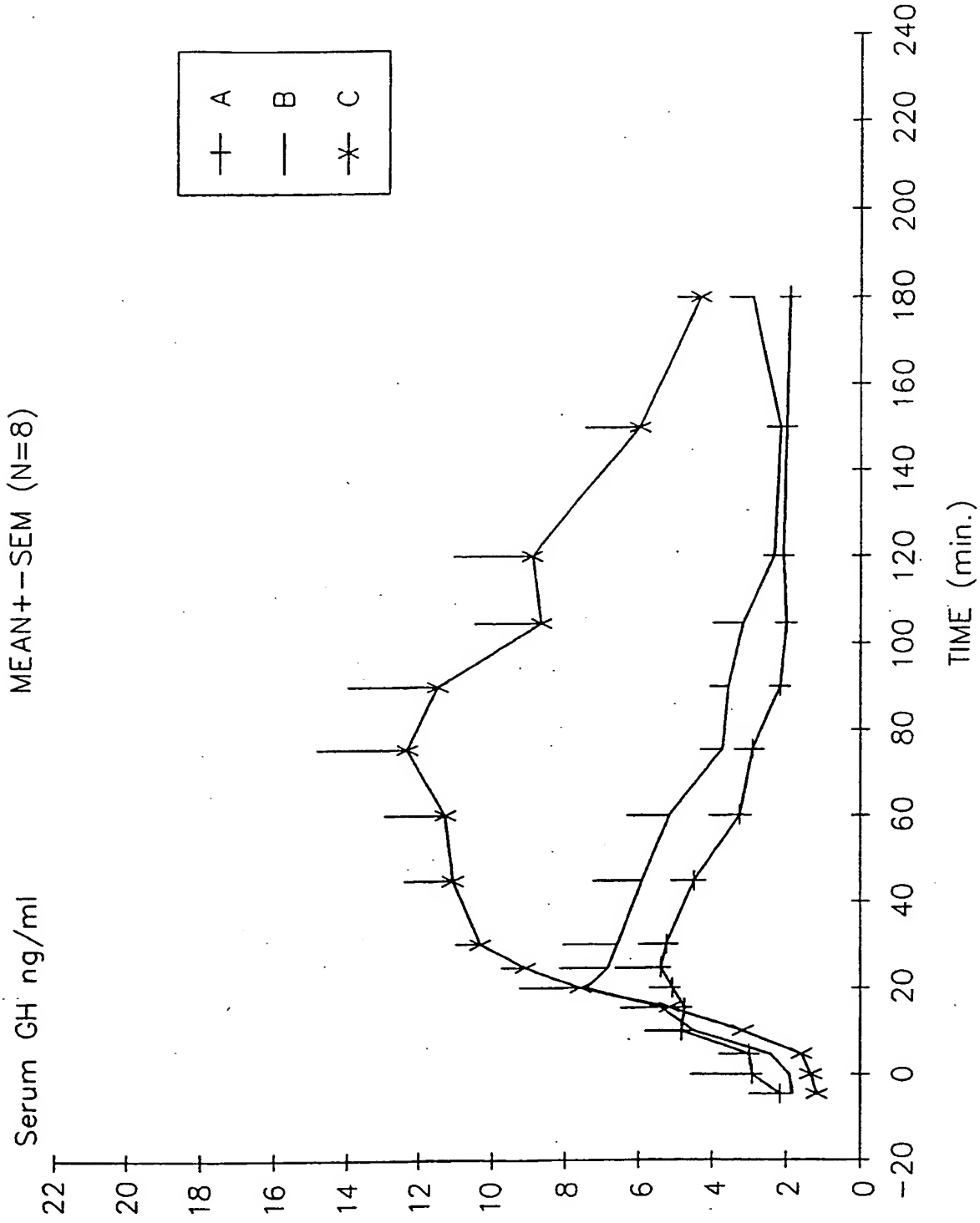

A
B
C
MEAN+-SEM (N=8)
Serum GH ng/ml
TIME (min.)
22
20
18
16
14
12
10
8
6
4
2
0
-20
0
20
40
60
80
100
120
140
160
180
200
220
240

Fig. 2

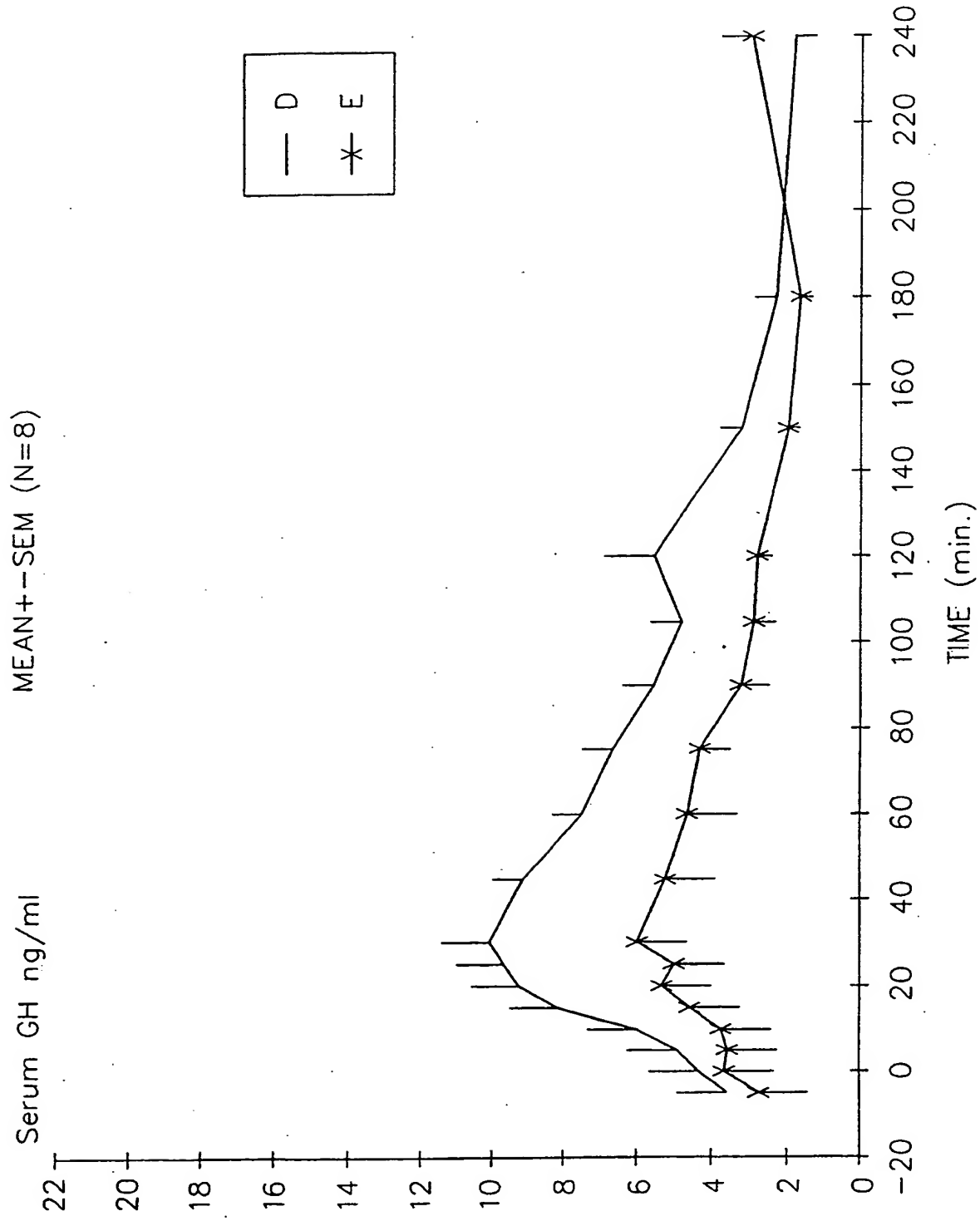

D
E
MEAN+-SEM (N=8)
Serum GH ng/ml
TIME (min.)
22
20
18
16
14
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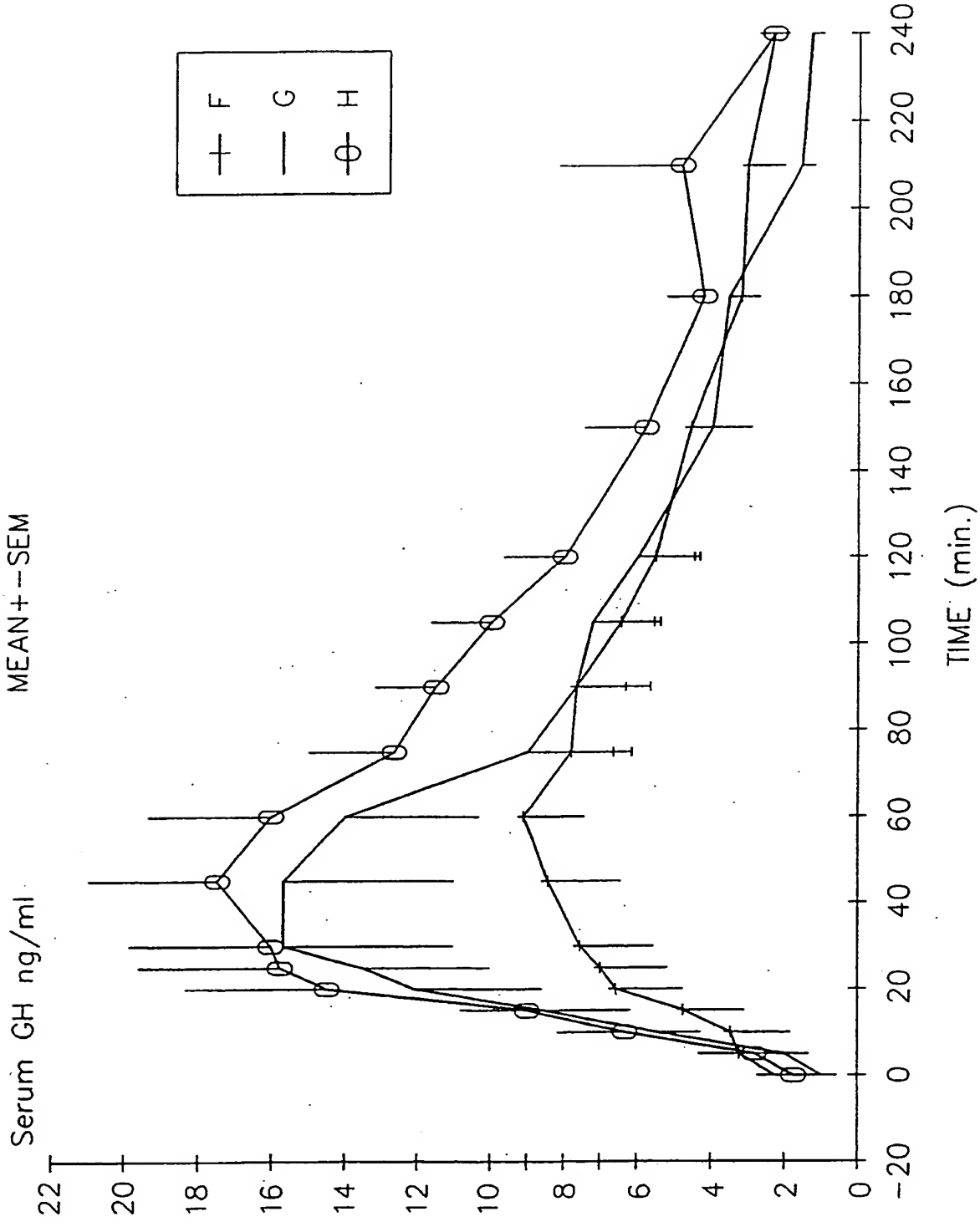
Fig. 3


F
G
H
MEAN+-SEM
Serum GH ng/ml
TIME (min.)
22
20
18
16
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12
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Fig. 4

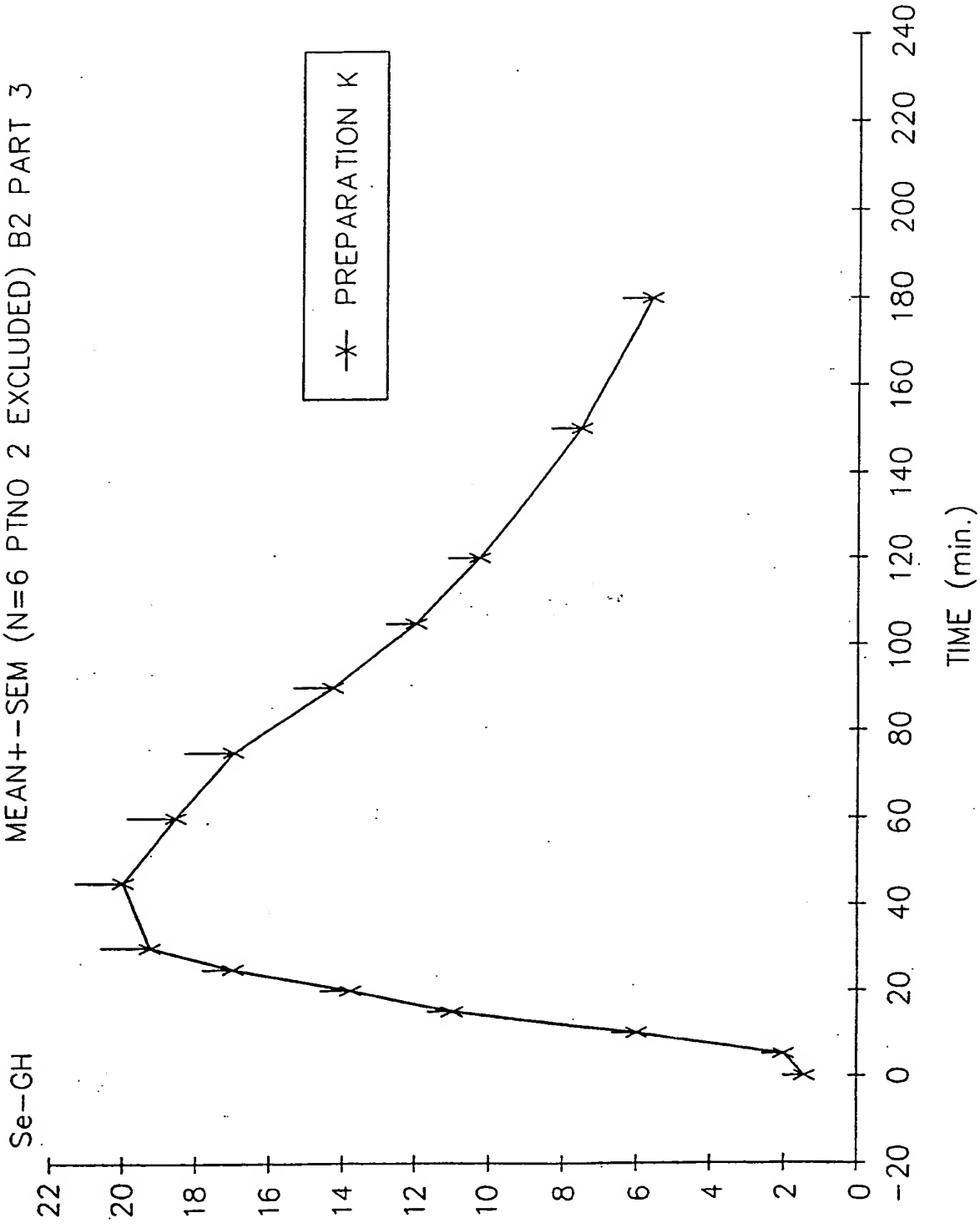
MEAN+-SEM (N=6 PTNO 2 EXCLUDED) B2 PART 3
PREPARATION K
Se-GH
22
20
18
16
14
12
10
8
6
4
2
0
-20
0
20
40
60
80
100
120
140
160
180
200
220
240
TIME (min.)

Fig. 5

INTERNATIONAL SEARCH REPORT

International Application No PCT/DK 92/00084

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: A 61 K 9/72, 9/14

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	US, A, 4613500 (SUZUKI ET AL) 23 September 1986, see the whole document --	1-8
A	WO, A1, 9201440 (RIJKSUNIVERSITEIT TE LEIDEN) 6 February 1992, see the whole document --	1-8
A	WO, A1, 8809163 (COSMAS-DAMIAN LIMITED) 1 December 1988, see the whole document --	1-8
A	EP, A2, 0200383 (ELI LILLY AND COMPANY) 10 December 1986, see the whole document --	1-8

---

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
29th June 1992	1992 -07- 02
International Searching Authority	**Signature of Authorized Officer**
SWEDISH PATENT OFFICE	Anneli Jönsson

Form PCT/ISA/210 (second sheet) (January 1985)

International Application No. PCT/DK 92/00084

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 9-11, because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the the claims. It is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additonal fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional seach fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/DK 92/00084

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the Swedish Patent Office EDP file on 29/05/92
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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A- 4613500	86-09-23	EP-A-B-	0122036	84-10-17
		JP-A-	59163313	84-09-14
		JP-B-	62037016	87-08-10
WO-A1- 9201440	92-02-06	NL-A-	9001681	92-02-17
WO-A1- 8809163	88-12-01	AU-B-	614290	91-08-29
		AU-D-	1793188	88-12-21
		EP-A-	0391896	90-10-17
		GB-A-	2231495	90-11-21
		JP-T-	2503915	90-11-15
EP-A2- 0200383	86-12-10	AU-B-	584184	89-05-18
		AU-D-	5606386	86-10-23
		JP-A-	62207221	87-09-11

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